Original Article

ROLE OF ENRICHED MEDIA IN BACTERIAL ISOLATION FROM SEMEN AND EFFECT OF MICROBIAL INFECTION ON SEMEN QUALITY: A STUDY ON 100 INFERTILE MEN

Orji Ikechukwu[1], Ezeifeka George[2], Amadi Ekperechi Sabinus[3], Okafor Florence[4]

ABSTRACT

Objective: The study was undertaken to determine the isolation potential of solid media with and without pre-enrichment as well as the possible influence of microbial infection on semen quality.

Methodology: Semen samples of 100 infertile men were cultured on solid media with and without complementation with liquid media culture (pre-enrichment) respectively. The cultured plates were examined macroscopically and the colonies identified. The sperm count, motility and morphological characteristics of the semen samples were also determined.

Results: The bacteria recovered from both cultural methods includes Staphylococcus aureus, *Staphylococcus epidermidis*, *ß-haemolytic streptococci*, *á-haemolytic streptococci, Escherichia coli*, *Proteus* and *Klebsiella* species. There was a significant increase in bacterial isolation when solid media culture was pre-enriched than when the former was used alone ($p<0.05$). There was no significant relationship between bacteria isolation and ranges of total sperm count, sperm motility, morphological abnormalities and age groups studied ($p<0.05$).

Conclusion: Pre-enriched solid media is a more efficient technique for optimal bacterial isolation from seminal fluid.

KEYWORDS: Semen, Pre-enrichment, Culture, Abakaliki.

Pak J Med Sci October - December 2007 (Part-II) Vol. 23 No. 6 885-888

INTRODUCTION

The isolation of microorganisms in seminal fluid especially of infertile men has been widely reported.[1-4] While the exact role of microbial infection in the aetiology of infertility is not very certain owing to the limitations in diagnostic criteria and asymptomatic nature of infection[5] some possible effect on the properties of seminal fluid associated with fertility has been suggested.[6,7]

In a comparative study among fertile and infertile population, fertile men were identified to have significantly fewer positive cultures than the infertile population.[8] Although microbial infection has been linked with infertility problem in a number of studies.[7,9,10] Gregoriou and co-workers[2] did not observe any significant effect of bacteriospermia on sperm quality.

The present study evaluates the isolation potential of solid media with and without pre-enrichment, as well as the possible effect of microbial infection on the quality of spermatozoa.

1. Mr. Orji Ikechukwu
Dept. of Medical Microbiology, Ebonyi State University Teaching Hospital Abakaliki, Nigeria.
2. Prof. Ezeifeka George,
Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka – Nigeria.
3. Dr. Amadi Ekperechi Sabinus,
Dept. of Applied Microbiology, Faculty of Applied and Natural Sciences,Ebonyi State University, P.M.B, 53 Abakaliki, Ebonyi State, Abakaliki – Nigeria.
4. Mrs. Okafor Florence,
Dept. of Medical Microbiology, Ebonyi State University Teaching Hospital, Abakaliki – Nigeria.

Correspondence

Dr. Amadi Ekperechi Sabinus,
E-mail: amadies2001@yahoo.com

* Received for Publication: March 24, 2007
* Revision Received: May 14, 2007
* Revision Accepted: September 27, 2007

MATERIALS AND METHODS

Specimen Collections: One hundred semen samples were collected from patients attending the infertility clinic of the Ebonyi State University Teaching Hospital, Abakaliki. Before specimen collection, patients were instructed to abstain from sex for at least three days. They were also requested to pass urine first and then wash and rinse hands and penis before the samples were collected.[11] The specimens were obtained by masturbation and ejaculated into clean wide-mouthed 15ml sterile plastic vials.

Determination of sperm count, motility and morphological characteristics: These were determined by microscopic examination of 10µl of properly mixed semen samples using the technique of WHO.[11]

Bacterial Isolation from Semen: Solid media plates of blood agar, chocolate agar, crystal violet blood agar, sodium chloride-nutrient agar and MacConkey agar were inoculated by surface plating with 0.1ml of semen samples. Similarly, 0.1ml each of the specimen were inoculated into culture bottles containing 20ml of Brain heart infusion broth and Tetrathionate medium. The plates and bottles were incubated aerobically at 37^0C for 24 hours. Replicate of the inoculations were made and incubated at increased carbondioxide.

Overnight cultures from Brain heart infusion broth and Tetrathionate medium were respectively sub cultured into the five solid media and incubated aerobically as previously stated. The respective culture plates were examined macroscopically and the colonies identified using standard methods.[12,13]

Table-I: Prevalence of Bacterial Isolates from Solid Media, with and without enrichment

Isolates	*Solid media without enrichment*	*Solid media with enrichment*
S. aureus	16 (32%)	33 (37.1%)
S. epidermidis	1(2%)	3(3.4%)
ß- haemolytic Streptococci	16(32%)	25(28.1%)
á- haemolytic Streptococci	9(18%)	10(11.2%)
E. coli	3(6%)	8(8.9%)
Proteus Species	3(6%)	7(7.9%)
Klebsiella Species	2(4%)	3(3.4%)
TOTAL	50	89

RESULTS

The result of the study showed that out of the 100 semen samples cultured in the solid media with enrichment, 89 yielded bacterial growths. *Staphylococcus aureus* (37.1%) constituted the highest bacterial isolate while *Staphylococcus epidermidis* (3.4%) and *klebsiella* species (3.4%) were the least (Table-I). However, out of the 100 semen samples cultured in solid media without prior enrichment, 50 yielded bacteria growth. *S. aureus* and ß – haemolytic streptococci were the highest bacterial isolates (32%) while *S. epidermidis* (2%) was the least (Table-I). The prevalence rate of isolation was highest in total sperm count of the range 0- 20 million per millilitre (58.4%) while the least was among semen samples with total sperm count range of 61 – 80 million per millilitre (Table-II). Also semen samples with sperm motility of the range 30- 40% yielded the highest number of bacterial growth while those

Table-II: Prevalence of bacterial isolates in relation to different ranges of total sperm count *(x10⁶/ml).*

	Ranges of Total Sperm Count (x10⁶/ml)						
Organisms	0-20	21-40	41-60	61-80	81-100	101-120	21-140
S. aureus	23(44.2%)	2(20%)	4(36.4%)	0(0.0%)	2(33.3%)	1(25%)	1(25%)
S. epidermidis	1(1.9%)	0(0.0%)	1(9.1%)	0(0.0%)	1(16.7%)	0(0.0%)	0(0.0%)
ß-haem. Strep.	13(25%)	6(60%)	2(18.2%)	1(50.0%)	0(0.0%)	3(75%)	0(0.0%)
á-haem. strep.	3(5.8%)	0(0.0%)	2(18.2%)	0(0.0%)	3(50.0%)	0(0.0%)	2(50%)
E. coli	6(11.5%)	1(10%)	1(9.1%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Proteus spp	5(9.6%)	0(0.0%)	1(9.1%)	0(0.0%)	0(0.0%)	0(0.0%)	1(25%)
Klebsiella spp	1(1.9%)	1(10%)	0(0.0%)	1(50%)	0(0.0%)	0(0.0%)	0(0.0%)
TOTAL	52(58.4%)	10(11.2%)	11(12.4%)	2(2.3%)	6(6.7%)	4(4.5)	4(4.5%)

Table-III: Prevalence of bacterial isolates in relation to different ranges of sperm motility (%)

	Ranges of Sperm Motility (%)			
Organisms	0-20%	30-40%	50-60%	70-80%
Staphylococcus aureus	6(31.6%)	11(37.9%)	10(41.7%)	6(35.3%)
Staphylococcus epidermidis	0(0.0%)	2(6.9%)	1(4.2%)	0(0.0%)
ß–haemolytic Streptococcus	8(42.1%)	6(20.7%)	5(20.8%)	6(35.3%)
á-haemolytic Streptococcus	1(5.3%)	4(13.8%)	3(12.5%)	2(11.8%)
Escherichia coli	2(10.5%)	3(10.3%)	1(4.2%)	2(11.8%)
Proteus Species	2(10.5%)	3(10.3%)	2(8.3%)	0(0.0%)
Klebsiella Species	0(0.0%)	0(0.0%)	2(8.3%)	1(5.9%)
TOTAL	19(21.3%)	29(32.6%)	24(27%)	17(19.1%)

within the range 70- 80% motility yielded the least (Table-III).

The highest prevalent rate of 46.1% was recorded for spermatozoa abnormality of the range 30 -40% followed by 50 - 60%, 70 – 80% and then 0 – 20% respectively (Table-IV). Also, the highest bacterial isolation was made from semen samples from individuals of age range 31-40 years while the least was among those within the age bracket 51–60 years (Table-V).

DISCUSSION

Staphylococcus aureus was the highest prevalent bacteria isolated in the study (37.1%). This is in line with the reports from previous studies.[3,7] Other isolates including *Streptococcus epidermidis*, *Escherichia coli, Proteus* species, ß-haemolytic Streptococcus, á-haemolytic streptococcus and *Klebsiella* species recovered in the study have also been reported by other workers.[1,3,7]

Of particular significance however, were haemolytic streptococcus, *Proteus* species and *Escherichia coli*, which had been reported to possess *in vitro* spermicidal activity.[9] Similarly 20.8% of infertility cases in 504 men studied in Nigeria were attributed to bacterial infection.[10] Further, poorly treated venereal diseases were identified as one of the prevalent causes of azoospermia in Nigeria.[14] Previous studies have also revealed that fertile men had significantly fewer positive cultures than the infertile population.[8] It does therefore appear that bacterial infections are probably involved in the infertility problem in Abakaliki.

That *S. epidermidis* constituted 2 – 3% of the overall bacterial isolates in this study was rather low compared with previous study[15] in which the organism accounted for 81.9% of the bacterial isolates. The reason for the variation was not immediately apparent. However, the prevalence of á-haemolytic streptococcus as recorded in this study (11 – 18%) is in conformity with prior work[15] in which the organism accounted for 18.1% of bacterial isolation.

There was a significant increase in the isolation of each bacteria when solid media culture of semen was complemented with liquid media culture (pre-enrichment) than when the former was used alone. Thus, the use of solid media alone in semen culture, which has been

Table-IV: Prevalence of bacterial isolates in relation to different ranges of morphological abnormality (%).

Organisms	*Ranges of Morphological Abnormality (%)*			
	0 – 20%	30 – 40%	50 – 60%	70 – 80%
Staphylococcus aureus	2(40%)	12(29%)	17(45.9%)	2(33.3%)
Staphylococcus epidermidis	0(0.0%)	2(4.9%)	1(2.7%)	0(0.0%)
ß-haemolytic Streptococci	3(60.0%)	10(24.4%)	9(24.3%)	3(50%)
á-haemolytic Streptococci	0(0.0%)	8(19.5%)	2(5.4%)	0(0.0%)
Escherichia coli	0(0.0%)	3(7.3%)	5(13.5%)	0(0.0%)
Proteus Species	0(0.0%)	3(7.3%)	3(8.1%)	1(16.7%)
Klebsiella Species	0(0.0%)	3(7.3%)	0(0.0%)	0(0.0%0
TOTAL	5(5.6%)	41(46.1%)	37(41.6%)	6(6.7%)

Table-V: Prevalence of bacterial isolates in relation to different age groups.

Organisms	*Age Group Range*			
	20 - 30	31 – 40	41 – 50	51 – 60
Staphylococcus aureus	8(36.4%)	17(38.6%)	6(31.6%)	2(50.0%)
Staphylococcus epidermidis	0(0.0%)	2(4.5%)	1(5.3%)	0(0.0%)
ß –haemolytic Streptococci	6(27.3%)	8(18.2%)	9(47.4%)	2(50.0%)
á-haemolytic Streptococci	5(22.7%)	5(11.4%)	0(0.0%)	0(0.0%)
Escherichia coli	1(4.5%)	5(11.4%)	2(10.5%)	0(0.0%)
Proteus Species	1(4.5%)	5(11.4%)	1(5.3%)	0(0.0%)
Klebsiella Species	1(4.5%)	2(4.5%)	0(0.0%)	0(0.0%)
TOTAL	22(24.7%)	44(49.4%)	19(21.3%)	4(4.5%)

the usual practice in hospitals and laboratories in Nigeria, might have been limiting the determination of the actual microbial content of semen. The influence of centrifugation on the enhancement of semen culture has been reported.[4]

There was no significant relationship between bacterial isolation and range of total sperm count, sperm motility, morphological abnormality and the different age groups as observed in this work ($p \leq 0.05$). This is consistent with the result of previous studies in which asymptomatic bacteriospermia in the semen was found not to significantly affect the sperm count, motility and morphological features of spermatozoa.[2,17]

However, it has been stated that the presence of bacteria in semen may affect fertility in several ways including damage of spermatozoa, hampering their motility, altering the chemical composition of the seminal fluid.[1] This study therefore advocates for the adaptation of pre-enriched solid media for optimal isolation of bacteria from semen, especially in the developing nations where advanced techniques are unavailable.

REFERENCE

1. Mogra N, Dhruva A, Kothari LK. Non-specific seminal infection and male infertility: A bacteriological study. J Post Grad Med 1981;27(2):99-104.
2. Gregoriou O, Botsis D, Papadias K, Kassanos D, Liapis A, Zourlas PA. Culture of Seminal Fluid in Infertile men and relationship to semen evaluation Int. J Gynaecol Obstet 1989;28(2):149-53.
3. Merino G, Carranza-Lira S, Murrieta S, Rodriguez L, Cnevas E, Moran C. Bacterial infection and semen characteristics in infertile men. Arch Androl 1995;35(1):43-7.
4. Villanueva-Diaz CA, Flores-Rejes GA, Beltran-Zuniga M, Echavarria-Sauchez M, Ortiz-Ibarra FJ, Arredondo-Garcia JL. Bacteriospermia and Male infertility: A method for increasing the sensitivity of semen culture. Int J Fertile Womens Med 1999;44(4):198-203.
5. Purvis K, Christiansen E. Infection in the male reproductive tract: Impact, diagnosis and treatment in relation to male infertility. Int J Androl 1993;16:1-13.
6. Bukharin OV, Kuzimin MD, Ivanov IB. The role of the microbial factor in the pathogenesis of male in fertility. Zh Microbial Epidemio Immunobiol 2000;2:106-110.
7. Rodin DM, Larone D, Goldstein M. Relationship between semen culture, leukopermia and semen analysis in men undergoing fertility evaluation. Fertil Steril 2003;79 (Suppl. 3):1555-8.
8. Toth A, Lesser ML. Assymptomatic bacteri ospermia in fertile and infertile men. Fertil Steril 1981;36(1):88-91.
9. Swenson CE, Toth A, Toth C, Wolfgruber L, O'Leary WM. Asympotomatic bacteriospermia in fertile men. Andrologia 1980;12:7-11.
10. Osegbe DN, Amaku EO. The causes of male infertility in 504 consecutive Nigerian patients. Int Urol Nephrol 1985;17:349-58.
11. World Heath Organization (WHO). Laboratory Manual for the examination of human semen and semen-cervical mucus interaction 1984;pp 3-4.
12. Cheesbrough M. Collection, transport and examination of clinical specimens. Medical Laboratory Manual for Tropical Countries. University Press Cambridge 1984;(2):186-7.
13. Chessbrough M. District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press 2000;157-8.
14. Kuku SF, Osegbe DN. Oligo/azoospermia in Nigeria. Arch Androl 1989;22:233-8.
15. Busolo F, Zanchetta R, Lanzone E, Cusinato R. Microbial Flora in semen of assymptomatic infertile men. Androlgia 1984;16:269-75.
16. Amin SA, Hamdy ME, Ibrahim AK. Detection of Brucella Melitensis in semen using the PCR assay Vet. Microbiol 2001;83(1):37-44
17. Bussen S, Zimmermann M, Schlever M, Steck T. Relationship of bacteriological characteristics to semen indices and its influence on fertilization and pregnancy rates after IVF. Acta Obstet Gynecol Scand 1997;76(10):964-8.